NOTES ON THE MORPHOLOGY OF SOME ISOLATES OF PHYTOPHTHORA FROM AUSTRALIA

by L. GERRETTSON-CORNELL*

ABSTRACT. The diagnostic importance of most morphological characteristics of 26 isolates of *Phytophthora* from various parts of Australia on two agar media has been discussed in this paper. Of these 13 were *P. cinnamomi* Rands, 9 were *P. cryptogea* Pethyb. & Laff., and 4 were *P. cambivora* (Petri) Buism. *P. cambivora* appears to have been found only 5 times in this country.

A new technique for the production of sporangia was tested and has proved highly reliable. This study also showed the difficulty of identifying *P. cambivora* from its morphology in the absence of oogonia under the experimental conditions. For certain characteristics, *P. cambivora* appeared to be closer to *P. cryptogea* than to *P. cinnamomi*.

RÉSUMÉ. La valeur systématique des caractères morphologiques de 26 souches australiennes de *Phytophthora*, cultivées sur deux types de milieux de culture est discutée. 13 souches appartiennent à *P. cinnamomi* Rands, 9 à *P. cryptogea* Pethyb. et Laff., et 4 à *P. cambivora* (Petri) Buism. Une technique nouvelle, favorable à la production des sporanges est proposée. Malgré la difficulté d'identifier *P. cambivora* en l'absence d'oogones, l'espèce apparaît plus proche de *P. cryptogea* que de *P. cinnamomi*.

INTRODUCTION

The present work describes most of the obvious morphological features of a number of isolates of *Phytophthora cinnamomi* Rands, *P. cryptogea* Pethyb. and Laff., and *P. cambivora* (Petri) Buism. from various parts of Australia. The study is part of a more comprehensive research programme designed to review the diagnostic importance of each character for *Phytophthora* species in view of the existing variability and differences often established with other descriptions.

* Forestry Commission of New South Wales, Beecroft, N.S.W., 2119, Australie.

The genus *Phytophthora* is widespread in Australia (GERRETTSON-CORNELL, 1976). In New South Wales *P. cinnamomi* and *P. cryptogea* have been frequently isolated from forest soils. *P. cambivora* has been detected only 4-5 times in this continent and this accounts for the low number of isolates available for this study. A thorough study of the sexuality has not been carried out but whenever possible the compatibility type of each isolate has been determined.

MATERIALS AND METHODS

a) Origins of cultures

The origins of the cultures of *Phytophthora* used in this study are listed in Table 1. Their identity was assessed or confirmed by the author, prior to the beginning of this work, by using the keys of WATERHOUSE (1954, 1963), FREZZI (1950) and various other descriptions (TUCKER, 1931; GRENTE, 1961; CHITZANIDIS and KOUYEAS, 1970). Two of the cultures were originally identified by the donors as *P. drechsleri* Tucker but on the basis of their morphology could not be separated from *P. cryptogea*. This is in agreement with other reported findings (BUMBIERIS, 1974; SHEPHERD, 1978 a). They also failed to grow at 34-36° C, in contrast to the description for *P. drechsleri* by WATERHOUSE (1963) and hence have been considered to be synonymous with *P. cryptogea*.

b) Mycelial growth and sporangial formation

Two media were used :

1. Corn meal agar (CMA) (Oxoid, 17 g/L). This medium is particularly suitable for the production of coralloid hyphae in some *Phytophthora* species. For example, during this study it was noticed that the mycelial structure of *P. cinnamomi* can vary considerably between isolates and also with the type of substrate used but tends in most cases to produce a coralloid type of growth on CMA. Moreover, because the aerial mycelium of most *Phytophthora* species on this medium is usually lacking or very poor, this enables a clear vision of the colony by direct microscopical examination of the cultures in the plates.

2. 2% V-8 juice agar (Uncentrifuged, pH 4.5). This medium, like CMA, enables a direct examination of cultures under the microscope and is also very good for the production of chlamydospores, sporangia and oogonia. Moreover, none of the present keys for the genus *Phytophthora* has been based on this medium.

For each isolate on each medium, three plates were used. They were inoculated centrally with a disc of agar mycelium set upside-down and incubated in darkness at 25° ± 1°C. They were examined every two days during which time they were exposed to room light.

To induce the formation of sporangia, 3 plates for each *Phytophthora* isolate

TABLE 1. List of isolates of Phytophthora used

Culture No.	Species	Mating type	Area of origin	Donor	Host association
8	*P. cinnamomi*	A1	Ourimbah, N.S.W.	Author	*E. saligna*
36	"	A2	Eden, N.S.W.	Author	*E. sieberi*
93	"	A2	Sunny Corner, N.S.W.	Author	*P. radiata*
102	"	A2	Madden's Plain, N.S.W.	Author	*E. gummifera*
51	"	A2	Wyong, N.S.W.	Author	*E. paniculata*
103	"	A2	West Pennant Hills, N.S.W.	Author	*E. pilularis*
DAR 19881	"	A2	Mt. Kuringai, N.S.W.	J. Walker	*Pimelea rosea*
DAR 25999	"	A2	Kenthurst, N.S.W.	J. Walker	*Actiridia chinensis*
T240	"	A2	Waterfall Bay, Tas.	F. Podger	*E. obliqua* and *Pultenea*
3477	"	A2	Kuranda, Qld.	B. Brown	Rainforest soil
3392	"	A1	Kuranda, Qld.	B. Brown	Rainforest soil
SC 50	"	A1	Manjimup, W.A.	J. Titze	Unknown
SC 90	"	A2	Kirrup, W.A.	J. Titze	*E. marginata*
S 111	*P. drechsleri*	A2	Unknown, N.S.W.	J. Shepherd	Unknown
S 121	*P. cryptogea*	A2	Adelaide, S.A.	J. Shepherd	Garden soil
31	"	A2	Gerringong, N.S.W.	Author	*P. radiata*
91	"	A2	Sunny Corner, N.S.W.	Author	*P. radiata*
104	"	-	West Pennant Hills, N.S.W.	Author	*E. pilularis*
105	"	A2	West Pennant Hills, N.S.W.	Author	*E. pilularis*
P7	"	A2	Adelaide, S.A.	M. Bumbieris	Garden soil
DAR 17095	*P. drechsleri*	-	Glen Innes, N.S.W.	J. Walker	*Carthamus tinctoris*
DAR 24233	*P. cryptogea*	-	Adelaide, S.A.	J. Walker	*Malus sylvestris*
S 104	*P. cambivora*	A1	Unknown	J. Shepherd	Unknown
S 107	"	A2	Perth, W.A.	J. Shepherd	*Malus sylvestris*
82	"	A1	Wallaroo, N.S.W.	Author	*E. paniculata*
WA 1390	"	A1	Perth, W.A.	R.F. Doepel	*Pyrus communis*

- : Did not form oogonia.

of 1 week old colonies on 2% V-8 agar flooded with glass distilled water. Young cotyledons of *Eucalyptus sieberi* L. Johnson, surface sterilized with 70% ethanol for 5 to 10 seconds and then rinsed in sterile distilled water, were used. They were floated on the water with which cultures had been flooded. These plates were then covered with their lids and incubated at room temperature (18-30°C), under a 40 W Grolux lamp. This new method for inducing sporangia formation is the author's adaption of the technique of MARKS and KASSABY (1974) for the isolation of *P. cinnamomi* from soil. It has proved extremely reliable provided the following conditions are observed :

- The test is made at temperatures above 16°C, and preferably between 20° and 25°C, either under a Grolux lamp or in a room with natural lighting plus 40 W, 3500° K fluorescent lamps. Exposure to light is either constant or for 8-10 hours a day.

- Not less than 10-15 cotyledons per plate of ca. 9 cm in diameter should be used.

- Young cotyledons, preferably between 2 and 3 weeks of age are used. At the third to fourth week and particularly at the appearance of the first pair of leaves, the formation of sporangia may be markedly reduced, or ceases altogether.

- It may happen, sometimes, that the water in the plates stagnates which may inhibit the formation of sporangia. It is advisable in that case to change the water a couple of times after 24-36 hours from the beginning of the experiment.

The formation of sporangia for all isolates of *Phytophthora* tested was preceded by colonization of the tissues of the host cotyledons by the mycelium and a concomitant, gradual change in colour of the underside purple colour of the leaves to green in 1 to 3 days.

c) Terminology

BLACKWELL's (1949) terminology has been used with some minor additions, as follows :

- Swollen hypha. It is an hypha or part of it, usually a long segment that is markedly enlarged. It may either be uniformly or irregularly enlarged (GERRETTSON-CORNELL, 1979). In this category, the large branches of swollen coralloid hyphae (fig. 1 and 2) are also included.

- Coralloid hypha. Coralloid hypha are generally branches of first and second order and are irregularly shaped. A mycelium is coralloid if it is formed prevalently coralloid hyphae, either with or without swollen hyphae. Coralloid hyphae may be single in which case they may be either alternate or opposite or be arranged in verticils on the bearing hypha.

Coralloids hyphae may bear one and even more swelling and/or chlamydospores.

- Swelling and chlamydospore. A swelling is a comparatively small portion

of an hypha which is markedly enlarged to form a globose, subglobose, or irregularly shaped body. It can be either sessile, terminal or intercalary, single or in clusters. *P. cinnamomi*, particularly on V-8 agar, produces botryose, thin-walled, greyish to yellow-brownish swellings, either globose, subglobose or irregularly shaped. It is often difficult to distinguish these from the chlamydospores. Chlamydospores have a septum or septa (if they are intercalary) which separates them from the bearing hypha but this is not always easy to see.

d) Period of observation

Results of this study were collected over a period of observation of 2 weeks.

RESULTS

Mycelium

On CMA all cultures of *Phytophthora* exhibited a type of mycelium growth eminently submerged into the agar with nil or very poor aerial mycelium. Colonies of *P. cinnamomi* on this medium were entirely or almost entirely coralloid. Colonies of *P. cambivora* were similar to *P. cinnamomi* although they formed more swollen hyphae which were clustered at the edge of the colonies or within 2-3 cm round the inoculum. Large branches of markedly swollen coralloid hyphae were observed in all isolates of *P. cambivora* (fig. 1, 2). In the past, the author has also observed a few of these formations in some isolates of *P. cinnamomi* from Eden, N.S.W. The mycelium of *P. cryptogea* was composed entirely or almost entirely of tubular hyphae. However, isolates S121 and 104 showed the presence of many coralloid hyphae, particularly round the inoculum.

Terminal and/or sessile swellings were numerous in *P. cinnamomi* but they also occured to a lesser extent in *P. cambivora* and there were a few in two isolates of *P. cryptogea*. A few, small clusters of intercalary swellings of the net-like type of configuration (WATERHOUSE, 1963) were seen in four cultures of *P. cryptogea*. Very few chlamydospores were formed by all isolates of *P. cinnamomi* and none by *P. cryptogea* and *P. cambivora*. Neither sporangia nor organs of fusion were formed by these *Phytophthora* species on corn meal agar.

The results of the microscopical examination of 2-week-old cultures on V-8 agar (Table 2) showed that all isolates of *P. cinnamomi* formed abundant botryose swellings and chlamydospores (fig. 3, 4) whereas these were not observed in *P. cryptogea* and *P. cambivora*. Hence growth on V-8 constitutes an important test for the identification of *Phytophthora*.

Table 2 also includes the mean diameter and range of the chlamydospores of *P. cinnamomi* based on samples of 50 chlamydospores for each isolate. By analysis of variance some of these values were found to be significantly different at the 1% level which may indicate real difference among isolates

TABLE 3 Mycelium characteristics on 2% V8 agar

	CH	SH	S T	S I	S Cl	Cld (µm)	Sp	Of
P. cinnamomi								
8	+ +	+	++	-	++	27(17-53)	-	-
36	++	+	++	+	++	31(18-51)	-	-
93	++	++	++	+	++	33(10-56)	-	-
102	++	+	++	-	++	37(22-59)	-	-
51	++	[illegible]	++	+	++	35(18-49)	-	-
103	++	+	++	-	+	31(15-48)	-	-
DAR 19881	++	+	++	-	++	44(22-55)	-	-
DAR 25999	++	++	++	+	+	33(15-48)	-	-
T240	++	+	++	-	++	34(15-55)	-	-
3477	++	+	++	+	++	41(16-66)	-	-
3392	++	+	[illegible]	+	++	41(22-59)	-	-
SC50	++	+	++	+	+	34(15-48)	-	-
SC90	++	-	++	-	-	35(22-48)	-	-
P. cryptogea								
S111	+	+	-	+	-	-	-	-
S121	+	+	-	+	-	-	-	-
31	+	+	-	+	-	-	-	-
91	+	+	-	+	-	-	-	-
104	+	+	-	-	-	-	-	-
105	+	+	-	-	-	-	-	-
P7	+	-	-	+	-	-	+	-
DAR 17095	+	+	-	+	-	-	-	-
DAR 24233	+	+	-	+	-	-	-	-
P. cambivora								
S104	++	+	+	+	-	-	-	-
S107	++	++	+	-	-	-	-	+
[illegible]	++	++	+	+	-	-	-	-
WA 1390	++	++	+	-	-	-	-	-

- : none observed
++ : abundant
CH : coralloid hyphae
T : terminal and/or sessile
Cl : chlamydospores
Cld: chlamydospores dimensions
Of : organs of fusion
+ : scarce
[illegible] : swollen hyphae [illegible] : swellings
I : intercalary
Sp : sporangia

regarding this particular characteristic. This fact shed further doubt on the value of the character «size» in identification of species. Sporangia and oogonia were not formed by most isolates of *Phytophthora* on V-8 agar over the period of observation. Only one isolate of *P. cryptogea* (P 7) formed 4 sporangia.

TABLE 3. Morphological characteristics on 2% V-8, under water and sporangia production by the cotyledon method

	Sporangia formation	Sporophore branching A	B	C	Intercalary swellings	Chlamydospores
P. cinnamomi						
[illegible]	++	++	++	++	+	++
36	++	++	++	++	+	++
93	++	++	-	+	- to +	++
102	++	++	+	+	+	++
51	++	++	+	+	+	++
103	++	++	-	+	+	++
DAR 19881	++	++	+	+	+	++
DAR 25999	++	++	+	+	-	+
T240	++	++	+	+	-	++
3477	++	++	-	-	+	++
3392	++	++	+ to ++	++	+	+
SC50	++	++	-	++	+	++
SC90	++	++	+ to ++	++	- to +	+
P. cryptogea						
S111	++	++	++	+	++	-
S121	++	++	++	+	++	+
31	++	++	+	-	++	-
91	++	++	++	++	++	-
104	++	++	+	+	++	-
105	++	++	+	-	++	-
P7	++	++	++	+	++	-
DAR 17095	++	++	++	+	+	-
DAR 24223	++	++	++	++	++	-
P. cambivora						
S104	++	++	+	-	++	-
S107	++	+ to ++	-	++	++	+
82	++	++	+	-	++	-
WA 1390	++	++	+	+	- to +	-

A: Sporophore unbranched B: sympodial branching

C: Proliferation through the empty sporangium.

-: none observed +: scarce ++: abundant

Sporangia

All isolates of *Phytophthora* in this study formed abundant sporangia on eucalypt cotyledons and surrounding agar. *P. cryptogea* produced them more rapidly and in the greatest quantity.

In *P. cryptogea* and *P. cambivora*, at the edge of cotyledons, large clusters of intercalary swellings of the net-type of configuration were also formed (fig. 5, 6, 7, 8). The diameter of these swellings reached 31μm. These formations in the two species looked exactly the same. A few (3) swellings in S 121 and S 107 exhibited dividing septa and have by definition to be regarded as chlamydospores. Isolate WA 1390 differed from the other isolates of this species in that it formed only one group of intercalary swellings on one plate. The similarity between *P. cryptogea* and *P. cambivora* with regard to this characteristic has already been reported (GERRETTSON-CORNELL, 1979). Under the conditions of the experiment, *P. cinnamomi* formed large clusters of botryose swellings and chlamydospores. On one occasion however a net-like type of configuration was observed inside one of these large groups. It was clearly observed to have originated by proliferation from each single swelling or chlamydospore rather than being an enlargement at more or less regular intervals of the same hypha.

Sympodial branching of the sporophore from below and proliferation through the empty sporangium were observed in most isolates of *Phytophthora*. Of the sympodial branching both close and the lax type occured, the close type being more frequently observed in *P. cryptogea*. The close type also occurred in *P. cinnamomi* and *P. cambivora*. In isolate 3477 (*P. cinnamomi* from Queensland) no branching of the sporophore was ever seen. As Table 3 clearly indicates, branching of the sporophore cannot be regarded as a distinctive character of identification.

Results of this work do not seem to be consistent with NEWHOOK's et al. (1978) new key of the genus *Phytophthora* on this particular aspect.

One hundred sporangia were selected for measurement from each isolate of *Phytophthora*. The dimensions of these sporangia are presented in Table 4.

There were no data for isolate S 107 since this fungus died and could not be replaced. However, it had previously been observed to be able to form sporangia with the cotyledon method and large clusters of intercalary swellings. Sporangia of all isolates of *Phytophthora* of this study were non-papillate ($< 2\mu$m) and had a wide pore ($> 7\mu$m).

The analysis of variance of the sporangial parameters showed the existence of highly significant ($p > 0.01$) difference between and within each species of *Phytophthora* for these characteristics. Variation in size was also observed in one isolate of *P. cambivora* whose sporangia were subsequently found to measure 38 x 27μm and 74 x 50μm. This seems to be consistent with the suggestion of BUMBIERIS (Pers. Comm.) that the first sporangia formed are sometimes different in size from those produced later. Further, the conditions of

TABLE 4. Mean value and range of length, breadth, Length/Breadth, and pore diameter of the Sporangia of Phytophthora.

Species	Isolate No.	Length (L) (µm)	Breadth (B) (µm)	L/B	Pore diameter (P) (µm)
P. cinnamomi	[illegible]	50 (24-82)	35 (21-48)	1.4 (1.1-2.1)	9 (5 -12)
	36	52 (22-92)	35 (18-53)	1.5 (1.2-1.9)	9 (5-15)
	93	45 (24-89)	31 (17-56)	1.5 (1.1-1.8)	10 (7-15)
	102	61 (40-73)	46 (33-55)	1.3 (1.1-1.8)	10 (6-15)
	51	52 (33-62)	39 (26-51)	1.3 (1.2-1.7)	10 (5-15)
	103	57 (26-73)	46 (22-55)	1.3 (1.1-1.7)	10 (5-15)
	DAR19881	62 (29-81)	43 (27-55)	1.4 (1.1-2.1)	12 (5-18)
	DAR25999	55 (40-77)	39 (27-51)	1.4 (1.1-2.3)	[illegible] (4-15)
	T240	54 (37-81)	38 (26-55)	1.4 (1.2-1.8)	10 (7-15)
	3477	51 (31-62)	38 (29-62)	1.4 (1.2-1.6)	10 (5-15)
	3392	57 (48-70)	43 (33-53)	1.3 (1.2-1.5)	10 (6-15)
	SC50	48 (31-71)	33 (26-44)	1.5 (1.1-2.2)	10 (5-15)
	SC90	56 (37-77)	38 (26-48)	1.5 (1.2-1.7)	10 (6-15)
P. cryptogea	S111	49 (27-65)	36 (22-45)	1.4 (1.1-1.5)	9 (8-12)
	S121	48 (37-66)	31 (22-38)	1.6 (1.1-2.2)	11 (8-15)
	31	47 (33-73)	32 (26-40)	1.5 (1.1-2.5)	9 (5-15)
	91	49 (26-66)	33 (15-44)	1.5 (1.1-2.0)	9 (7-11)
	104	50 (34-62)	34 (22-44)	1.5 (1.2-1.8)	10 (8-12)
	105	50 (26-62)	38 (18-48)	1.3 (1.1-1.6)	10 (6-15)
	P7	51 (44-66)	37 (26-44)	1.4 (1.1-1.8)	10 (5-13)
	DAR17095	47 (29-70)	29 (18-40)	1.7 (1.1-2.6)	11 (5-18)
	DAR24233	52 (40-66)	37 (29-48)	1.4 (1.2-1.7)	10 (5-15)
P. cambivora	S104	47 (22-63)	31 (17-41)	1.5 (1.2-2.3)	10 (7-14)
	82	74 (48-95)	50 (33-66)	1.5 (1.2-2.0)	14 (11-18)
	WA1390	55 (44-77)	42 (29-55)	1.4 (1.1-1.7)	13 (9-18)

experiment influence enormously the character size (LEONIAN and GEAR, 1929; TUCKER, 1931; CHITZANIDIS and KOUYEAS, 1970). Sporangia of all isolates of *P. cinnamomi*, *P. cryptogea* and *P. cambivora* in this study were non caducous and exhibited no pedicel or this was irregular and very difficult to measure. Of these two characters, sporangium caducity would seem to be more stable a character than pedicel length. On the other hand AL-HEDAITHY & TSAO (1979) in a study with isolates of 7 species of *Phytophthora* including *P. cinnamomi*, clearly showed that the degree of caducity alone has questionable diagnostic value and that it should be determined only if both the degree of caducity and uniformity in pedicel length are considered.

Sporangial germination

Over the period of observation (4-5 days from the moment cotyledons were floated on the water covering the cultures) only the indirect type of germination of the sporangium (i. e. by zoospores) was observed for *P. cinnamomi* and *P. cambivora*. In *P. cryptogea* the indirect type was also frequent but some sporangia (particularly those with a vacuole) exhibited the direct germination, i. e. by germ tube. No vacuolated sporangia were seen to form zoospores which is consistent with previous findings (GERRETTSON-CORNELL, 1979). *P. cryptogea* showed four different types of zoospore release. Zoospores were formed most frequently within the sporangium and spewed out singly, in rapid succession. Quite often, as already observed in *P. cinnamomi* from Ourimbah State Forest (GERRETTSON-CORNELL, 1973), the sporangium expelled the whole protoplasm as a shapeless mass which broke into single units immediately after release. Less frequently the protoplasm was held at the mouth of the sporangium for a few seconds; from this, small portions of protoplasm separated in regular succession from it and gave rise to one or even two zoospores. On other occasions the sporangium expelled the protoplasm as a whole mass of individualised cells and this moved around until it broke into groups of elements stuck together and eventually into single units (GERRETTSON-CORNELL, 1979). On one occasion, three minutes elapsed from the moment this mass was ejaculated and its breakup into single zoospores. In *P. cinnamomi*, the first two types of zoospore formation were most frequently observed but the other two also occured. The second and third methods seemed to occur more frequently in *P. cambivora*.

Shape of the sporangium

Some of the final conclusions of a study designed to assess the importance of this character in the identification of *Phytophthora* spp. (GERRETTSON-CORNELL, 1980) are summarized. This work showed that although the presence of certain sporangial shapes can be more indicative of some species of *Phytophthora* than others, the same shapes can be found in the latter although to a lesser extent. Moreover, there are shapes which are common to the three species *P. cinnamomi*, *P. cryptogea* and *P. cambivora*. Consequently the sporangial shape cannot be considered a character of primary importance in the identification of *Phytophthora* but only as a supplementary test.

DISCUSSION

This study has shown the existence of some variability among isolates of the same species of *Phytophthora* for a few characteristics such as the size of the sporangia, the presence or absence of swellings. In general however, there was reasonable uniformity within each species. For example, on CMA all isolates of *P. cinnamomi* had a mycelium almost entirely coralloid. Other examples, under the experimental conditions, were the general lack of chlamydospores in *P. cambivora* and *P. cryptogea* on CMA and V-8 agar and the prevailing incapacity to form oogonia homothallically, consistently with other people's work (TUCKER, 1931; FREZZI, 1950; GRENTE, 1961; WATERHOUSE, 1963). This study also confirmed the occurence of homothallism in *P. cambivora*, in the same isolate A2 already described (GERRETTSON-CORNELL, 1977).

Formation of intercalary swellings in large clusters of a net-type configuration at the edge of cotyledons under water was confirmed in all isolates of *P. cambivora*. These swellings were identical to those formed by *P. cryptogea*.

For the first time, intercalary swellings were also observed within the botryose clusters of swellings in *P. cinnamomi*.

Colonies of *P. cinnamomi* and *P. cambivora* on CMA appeared very similar. The scanty aerial mycelium was formed from a few tubular hyphae whereas the substrate mycelium was coralloid. That of *P. cambivora* was formed of more flexuous hyphae and exhibited clusters of «markedly» swollen coralloid hyphae. However these may also be found in certain isolates of *P. cinnamomi*. More definite differences between *P. cinnamomi* and *P. cambivora* resulted on 2% V-8 agar where all isolates of *P. cinnamomi* produced botryose, yellowish-brownish thin-walled swellings and/or chlamydospores whilst *P. cambivora* could only form, at times, a few, sparse hyaline-greyish swellings.

The mycelium of *P. cryptogea* on CMA varied from being formed almost exclusively of tubular hyphae or tubular hyphae with some coralloid hyphae. On V-8 agar, the hyphae were prevalently tubular. The presence of few, small clusters of intercalary swellings (in some isolates), the constant absence of sporangia and oogonia, on solid media, the formation at times of few sporangia, sparsely, non papillate, often with a central vacuole may be indicative of this species of *Phytophthora*. All isolates of *P. cryptogea* under water and in the presence of cotyledons were characterised by a «rich» production of clusters of intercalary swellings.

CONCLUSIONS

The morphology of the isolates of *Phytophthora* has been determined and despite the limitations imposed by the small number of *P. cambivora* examined, the following characteristics under the experimental conditions used are consi-

dered to be of primary importance for the identification of *P. cinnamomi*, *P. cryptogea* and *P. cambivora* :

1. Prevailing heterothallism, amphigenous antheridium and a prevailing lack of sporangia on solid media.
2. The oogonium wall, either smooth or bullate.
3. Lack of a papilla in the sporangium, i. e. apical hyaline thickening $<$ 3-3,5μm thick.
4. Pore diameter $>$ 7μm.
5. Sporangia non deciduous.
6. Ability to form botryose swellings or intercalary swellings directly on solid media or in the presence of water.
7. Presence or absence of chlamydospores.

The following characteristics are considered to be of lesser importance and are used as a supplementary test.

1. The texture of the mycelium on CMA.
2. The presence of particular predominant sporangial shapes and the frequency by which they occur.

Minimal taxonomical importance is attributed to the size of the sporangia because of their variability. Similarly, the branching of the sporophore and the method of release of the zoospores are features which appeared to be too erratic to be of any taxonomical value for the identification of these *Phytophthora* spp.

Under the conditions of experiment and by combining these observations on CMA and V-8 media with those of WATERHOUSE (1963), the following key for the identification of *P. cinnamomi*, *P. cryptogea* and *P. cambivora* is suggested.

KEY

- Sporangia and oogonia usually not formed in single culture. Sporangia formed in the presence of liquid media, non-papillate (papilla $<$ 3-3,5μm), pore size $>$ 7μm, non-deciduous. Oogonia formed heterothallically, antheridium always amphigynous.
  - Sparse (sessile or terminal) and «botryose» swellings, and chlamydospores formed. Mycelium chiefly coralloid on CMA. . . . . *P. cinnamomi*
  - Botryose swellings not formed, chlamydospores not formed.
    - Bullate as well as smooth oogonia formed in paired cultures with *P. cambivora* and *P. parasitica*, in particular. Mycelium, on CMA, rich in swollen, either coralloid or not, hyphae. Net-like clusters of intercalary swellings may be formed under water with or without cotyledons. Some sessile and/or terminal swellings may be present. Sporangia may exhibit a «markedly» round apex, they may even be conical with a pointed apex. . . . . *P. cambivora*

- Mycelium composed of tubular hyphae with or without coralloid hyphae. Net-like clusters of intercalary swellings formed with and (but only at times) without water. Sporangia readily formed under water, either obpyriform and/or of various other shapes, often vacuolated. . . . . *P. cryptogea*

Compared with WATERHOUSE (1963), the above key has the advantage of not using the character «size» of the sporangia as a preliminary and important discriminatory test. Further it refers to specific conditions of experimentation and includes some additional characteristics such as the ability of some isolates of *P. cambivora* on V-8 to form intercalary swellings under water with cotyledons (and even without in some cases). Larger variation in the branching of the sporophore was observed than that described by WATERHOUSE (1963) for the above three species of *Phytophthora*.

P. cinnamomi form oogonia when paired with the opposite compatibility type as well as with *P. cambivora* and *P. cryptogea*. Bullate oogonia in *P. cambivora* are formed, besides with its opposite type, by pairing it with *P. parasitica* and *P. cinnamomi* (WATERHOUSE, 1954, 1963; GRENTE, 1961; CHITZANIDIS and KOUYEAS, 1970; SHEPHERD, 1978 a, b). *P. cryptogea* yields oogonia with its opposite compatibility type and with *P. cinnamomi* A1 and A2. In particular the work by SHEPHERD (1978 a, b) provided detailed information on the mating behaviour of Australian isolates of *Phytophthora*.

This study has shown the immense difficulty of identifying *P. cambivora* when there is no possibility of mating it, and its identity could only be suspected from the general description of the mycelium. Furthermore *P. cambivora* showed to be closer to *P. cryptogea* than to *P. cinnamomi*. For example, they both failed to form chlamydospores and the typical botryose swellings of *P. cinnamomi*. At the same time both species were able to produce intercalary swellings in net-like structures. Other similarities between these two *Phytophthora* species with regard to the shape of the sporangium were observed (GERRETSSON-CORNELL, 1980).

There is chaos to some extent in the genus *Phytophthora*. WATERHOUSE's Key (1963), although of great importance in the past, suffers from some limitations in certain groups such as Nos 5 and 6. FREZZI's (1950) key, although more precise, is based only on 12 species of *Phytophthora*. The absence of species such as *P. cambivora* in it limits its application. Furthermore the existence of isolates with characteristics of one, two and even three species of *Phytophthora* recently established by the author in Australia adds new confusion to that already existing. These could be hybrids or even different strains of the same species or indicate that different sorts of grouping rather that the present scheme based on species alone could be adopted.

ACKNOWLEDGEMENTS

The author is indebted to Messrs. John WALKER, Biological and Chemical Research Institute, Rydalmere, N.S.W., D.W. EDWARDS, Group Leader, Biology and Mrs Marcia LAMBERT, Chemistry Section for helpful comments on this paper.

Mrs. Anne MUSH is also acknowledged for her statistical work. Those who gave isolates of *Phytophthora* and whom are listed in Table 1, are also kindly acknowledged.

BIBLIOGRAPHY

AL-HEDAITHY S.S.A. and TSAO D.H., 1979 - Sporangium pedicel length in *Phytophthora* species and the consideration of its uniformity in determining sporangium caducity. *Trans. Br. mycol. Soc.* 72 : 1-13.

BLACKWELL E., 1949 – Terminology in *Phytophthora*. *Mycological Papers* No 30. The Commonwealth Mycological Institute, Kew, Surrey, England.

BUMBIERIS M., 1974 – Characteristics of two *Phytophthora*. *Aust. J. Bot.* 22 : 655-660.

CHITZANIDIS A. and KOUYEAS H., 1970 – Notes on Greek species of *Phytophthora*. *Annls. Inst. Phytop. Benaki*, N.S. 9 : 267-274.

FREZZI M.J., 1950 - Las especies de *Phytophthora* en la Argentina. *Ministerio de Agricultura y Ganaderia*, Buenos Aires.

GERRETTSON-CORNELL L., 1973 – A preliminary study of the morphology of *Phytophthora cinnamomi* Rands from Ourimbah S.F. (Wyong.) *Inf. Bot. Ital.* 5 : 78-82.

GERRETTSON-CORNELL L., 1976 – Effect of different methods of water demineralization on the recovery of *Phytophthora cinnamomi* Rands by lupin baiting. *Phyton* 34 : 85-87.

GERRETTSON-CORNELL L., 1977– Preliminary results of a study of the sexuality in *Phytophthora cambivora* (Petri) Buis. *Phyton* 35 : 153-157.

GERRETTSON-CORNELL L., 1979 – Notes on the morphology of five isolates of *P. cryptogea* from Australia. Accepted for publication in *Phyton*.

GERRETTSON-CORNELL L., 1980 - Submitted for publication.

GRENTE J., 1961 – La maladie de l'encre du chataigner. *Ann. Epiphyties*, 12: 25-59.

LEONIAN L.H. and GEER H.L., 1929 - Comparative value of the size of *Phytophthora* sporangia obtained under standard conditions. *J. Ag. Res.* 39 : 293-311.

MARKS G.E. and KASSABY F.Y., 1974 - Detection of *Phytophthora cinnamomi* in soils. *Aust. For.* 36 : 198-203.

NEWHOOK F.J., WATERHOUSE G.M. and STAMPS D.J., 1978 – Tabular key to the species of *Phytophthora* de Bary : *Mycological Papers* No 143. The Commonwealth Mycological Institute, Kew, Surrey, England.

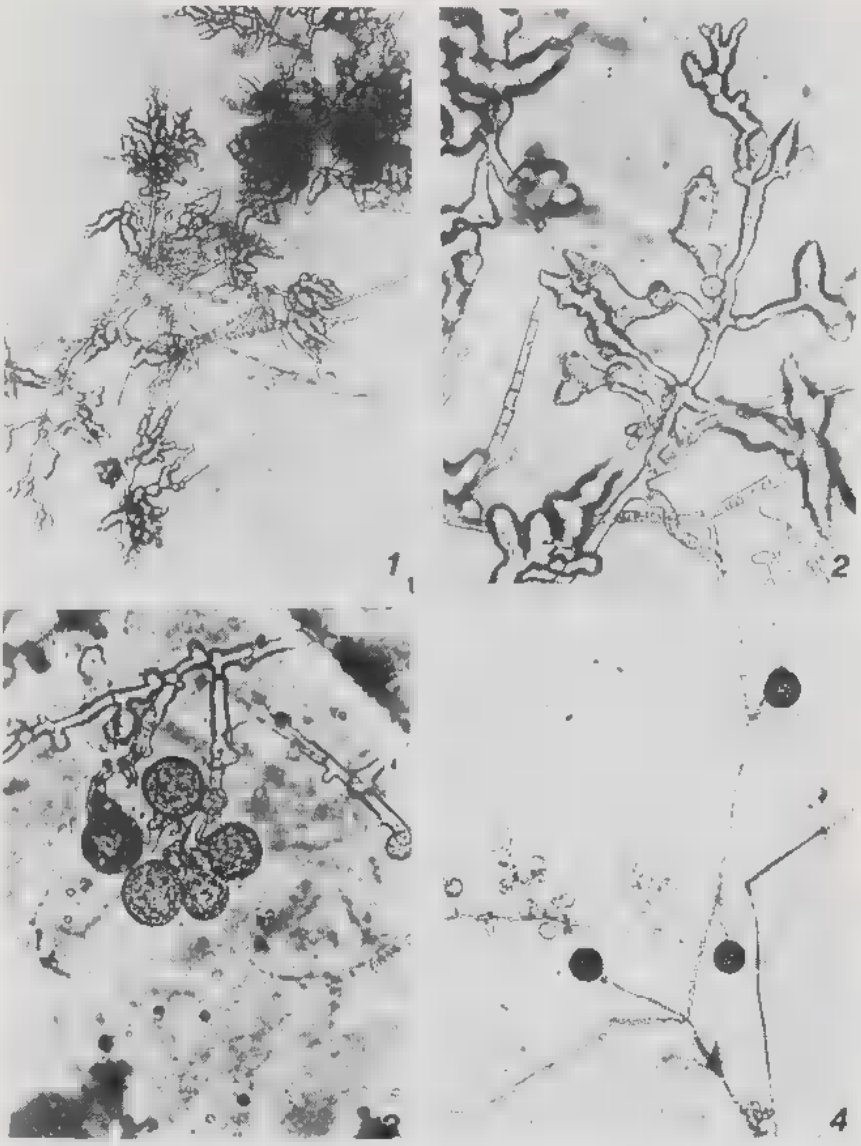
SHEPHERD C.J., 1978 a Mating behaviour of Australian isolates of *Phytophthora* species. I. Inter and intra-specific Mating. *Aust. J. Bot.* 26 : 123-138.

SHEPHERD C.J., 1978 b – Mating behaviour of Australian isolates of *Phytophthora* species. II. *Phytophthora cinnamomi*.

TUCKER C.M., 1931 – Taxonomy of the genus *Phytophthora* de Bary. University of Missouri, Columbia.

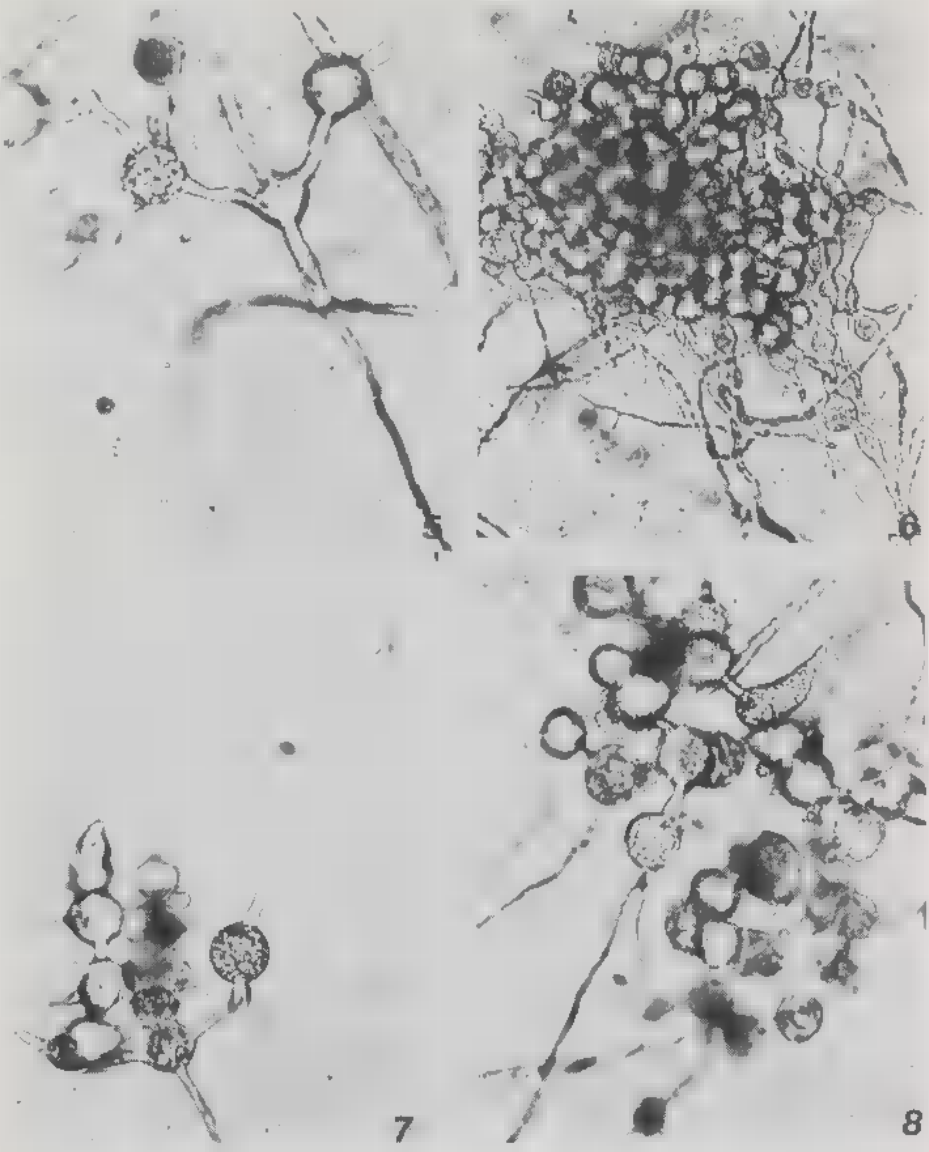
WATERHOUSE G.M., 1954 – Key to the species of *Phytophthora* recorded in the British Isles. *Mycologia* 57.

WATERHOUSE G.M., 1963 – Key to the species of *Phytophthora* de Bary. *Mycological Papers* No 92. Commonwealth Mycological Institute, Kew, Surrey, England.



Pl. I.

Fig. 1, 2 : *Phytophthora cambivora* ; clusters of swollen coralloid hyphae, x 275 and x 440. Fig. 3 : *P. cinnamomi* ; botryose swellings and chlamydospores, x 250. Fig. 4 : *P. cinnamomi* ; chlamydospores and «small» botryose swellings, x 125.



PL. II.

Fig. 5, 6 : *P. cryptogea* : intercalary swellings, x 440 and x 275. Fig. 7, 8 : *P. cambivora* : intercalary swellings, x 440.